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Genome Editing in Fruit Crops – A Review

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ABSTRACT: Conventional fruit and fruit tree breeding has improved consumer-driven traits like fruit size, yield, nutritional value, scent, and flavour while also introducing agronomic features like disease resistance. However, because of the long juvenility, genetic improvement through conventional breeding has been slow. Genome editing, a novel genetic improvement tool that can greatly accelerate the development of perennial crops, has been made possible by advancement in genomics and molecular biology. This article describes genome editing technologies, including CRISPR-C as system-based genome editing, and various applications of them in enhancing fruit crops. In addition, base editing, a more precise editing technique that has recently been emerged for enhancing fruit and nut crops will also be discussed.

Keywords: genome editing, fruit crops, CRISPR/Cas 9, applications.

INTRODUCTION

Fruit and nut crops have a major role in food and nutritional security as well as in the food production systems. Due to the growing global population and dietary preferences, a sustained increase in fruit and nuts production is required (Hwalla *et al*., 2016). Numerous important fruit and nut crop varieties with improved yield, quality, colour, and size attributes have been developed through conventional breeding. However, conventional breeding is slow and its outcomes are ambiguous in these perennial crops due to their lengthy juvenile stages, heterozygous character, and lack of connections between seedling and adult plants. Hence, conventional breeding techniques are insufficient and ineffective for producing variations quickly enough to meet shifting consumer demands, adapt to changing climatic conditions and address shifting socioeconomic variables like a shrinking labour force and rising energy cost (Chen *et al*., 2020).

The development of several genomic-based breeding tools that allow targeted and precise genetic alterations in crop plants has been made possible by the advancements in molecular biology and genomic research in plants. Genome editing is an emerging genomics-based technology that enables precise and targeted alterations in genome. The genetic improvement of perennial crops could be revolutionized by genome editing, which can greatly speed up varietal development. In this review article, we cover the genome editing technologies, CRISPR-Cas system-based genome editing and its potential applications in fruit and nut crops improvement.

Genome editing. Natural genetic changes have been happening in plants primarily as a result of random mutations and their fortunate selection that led to the development of the crops used today. Due to genetic alterations brought about by hybridizations and mutagenesis utilizing radiation or chemical agents, genetic variants improved genetically with the advent of contemporary breeding practices (Goulet *et al*., 2017). But the genetic modifications created are at uncertain locations across the genome, producing unpredictable results.

Genome editing technologies, which recently made it possible to make exact genetic modifications at certain loci, are those that allow for precise DNA editing (Hua *et al*., 2019). The mutations or modifications brought about by genome editing are similar to those that happen through natural or conventional breeding, but has more specificity (Cao *et al*., 2016; Tzftra *et al*., 2012). Fig. 1 illustrates the length of breeding cycles and generation time for fruit crops using genome editing technology.

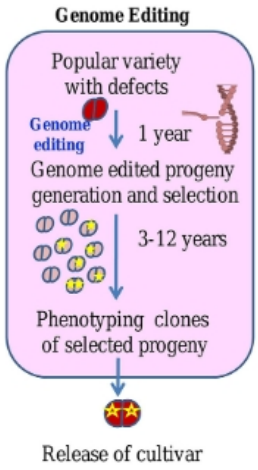

Fig 1. Schematic representation of the length of breeding cycles using genome editing in fruit crops.

Engineered endonucleases are used in genome editing to produce specific changes at targeted loci in the genome. There are different genome editing tools available, of which three have been utilized in fruit crops namely transcription activator-like effector nucleases (TALENs), CRISPR/Cas 9 and zinc finger nucleases (ZFNs) (Ghogare *et al*., 2020; Shukla *et al*., 2009; Zhang *et al*., 2013). TALENs have been used to enhance characteristics in various fruits and vegetables (Khan *et al.*, 2017), whereas ZFNs have been used to modify selectable markers in apple and fig (Peer *et* al., 2015). These systems have only been used to a limited extent in fruit crops, though, because of the complex construct design principles (Carroll, 2011). CRISPR/Cas 9 is the most widely used system to edit many fruit crops (Zhou *et al*., 2020). For example, the use of CRISPR/Cas 9 in tomato, banana, grapevine, papaya, watermelon and cocoa has increased tolerance to abiotic stress (De Toledo *et al*., 2016; Tian *et al*., 2018; Wang *et al*., 2017 and Yin *et al*., 2018). Using CRISPR/Cas 9, cultivars of tomato, cucumber, groundcherry, and kiwifruit have also been domesticated (Hu *et al*., 2017; Lemmon *et al*., 2018).

The basic principle behind technologies like ZFNs, TALENs, and CRISPR-Cas9 is that DNA sequences at the targeted loci are broken into double strands by the relevant endonucleases (DSBs), which are then repaired by one of the two DNA repair pathways, *i.e*., Nonhomologous end-joining (NHEJ) and homologous recombination (HR) in the cell. Without using a repair template that results in insertions or deletions in the repaired DNA, the NHEJ process achieves DSB repair. NHEJ mechanisms randomly generate mutations, which cannot be controlled. In contrast, the DSBs in the HR-based path way are repaired using a repair template that contains homologous sequences to the sequence that follows DSBs. The production of mutations in the genome can be precisely controlled using the HR process. However, the HR pathway is less effective at repairing DSBs than the NHEJ pathway (Lieber *et al*., 2010).

CRIPSR/Cas 9 based genome editing. CRISPR/Cas 9 system has shown to be the most effective and user friendly among the three genome editing technologies. Due to its improved targeting effectiveness, wide range of applications and simplicity of use, it has been widely used in genome editing (Doudna *et al*., 2014). The CRISPR/Cas 9 system is based on a nuclease (Cas 9) that recognizes the protospacer neighboring motif, a very short and common sequence (3–8 nt in length) (PAM). The nuclease is directed to a more precise target, which is a sequence complementary to the PAM, by the aid of a guide RNA (gRNA). The gRNA has a nucleotide length of 20. The CRISPR/Cas 9 system is more adaptive than other editing tools because gRNA can target several genes at once and is simpler to make than ZFNs and TALENs modules (Bortesi *et al*., 2016; Armario *et al*., 2019).

Since their discovery, the CRISPR-Cas system's potential genome editing applications have undergone a lot of important advancements. It has been found that distinct bacteria and archaeal cells have evolved CRISPR-Cas systems in diverse ways (Mohanraju *et al*., 2016). The Cas genes and the production of gRNA determine how these various CRISPR-Cas systems operate. Based on the composition of the effector genes, CRISPR-Cas genes are divided into Class 1 and Class 2. Class 1 has a complex of numerous effector proteins, whereas class 2 only has a single effector protein. Based on differences in how pre-crRNA is processed and the variety of domains found in the nuclease protein, these two primary groups are further divided into subclasses. CRISPR systems of types I, III, and IV are included in class 1, whereas CRISPR-Cas systems of types II, V, and VI are included in class 2 (Koonin *et al*., 2017). The type II and V of class 2 CRISPR-Cas system are found ideal for DNA editing, whereas type VI is used for RNA editing.

Recent advancements in the CRISPR-Cas9 system enable accurate and target-specific alteration of genomic regions and regulation of gene expression. Additionally, it can be used in a range of cells and organisms and is inexpensive. Thus, a revolution in genome alteration for novel biotechnological applications has been brought about by CRISPR-Cas9. Target genes regulating the desired target gene or locus are identified for CRISPR-Cas9-based genome editing, and guide RNAs (sgRNAs) are made to precisely direct the Cas9 endonuclease to the target gene or locus.sgRNAs are created by utilizing bioinformative tools, while taking into account potential off-targets in the genome. The co-expression of sgRNAs and Cas9 endonuclease in the transformed plants is made possible by the cloning of the sgRNA and Cas9 coding sequences into expression vectors. The transformed cells or plants are recognized using the selection or reporter markers, which are also a part of the genome editing vector. Verification of the alterations caused by genome editing is possible by sequencing the target gene in transformed plants. The CRISPR-Cas9 cassette can be deleted from sexually reproduced plants by segregations in the following generation to produce genome-edited plants free of transgenes (El-Mounadi *et al*., 2020).

However, it might not be able to follow the segregation to achieve the necessary genome-edited plants in perennial crops that are propagated via cloning, such as many fruit tree crops (Fan *et al*., 2020; Malnoy *et al*., 2016; Osakabe *et al*., 2018; Poovaiah *et al*., 2020; Woo *et al*., 2015). The plants produced in this manner are free of exogenous DNA sequences. Thus, fewer rigorous biosafety criteria are predicted for DNA/transgene-free genome editing utilizing CRISPR-Cas9. An illustration of fruit crop genome editing using CRISPR-Cas 9 technology (Fig. 2).

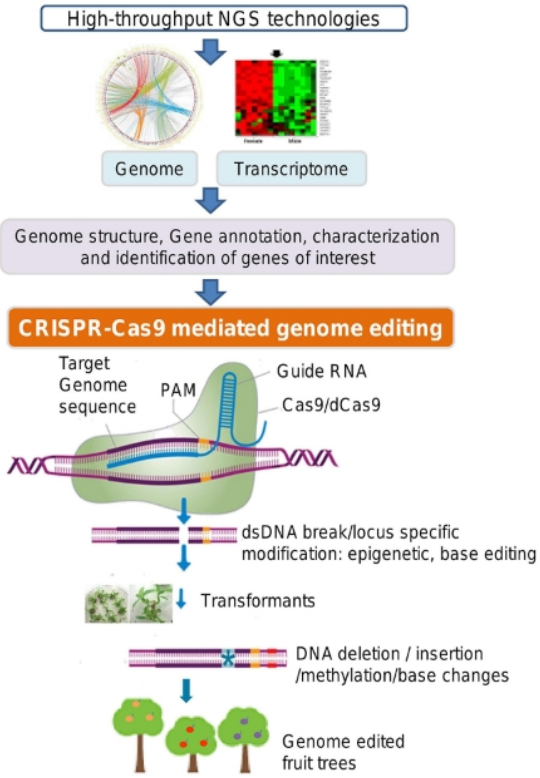

Fig. 2. Schematic representation of CRISPR-Cas 9 based genome editing in fruit crops.

Potential applications of CRISPR/C as 9 based genome editing in fruit crops. With the advancement in genomics and molecular biology, genome editing has been applied to several crops, including the perennial crops like fruits (Kamburova *et al*., 2017). Using the CRISPR/Cas system, the first genome editing was described in the sweet orange in 2014. List of genome editing studies in different fruit crops are given in Table 1. This strategy can be used to enhance traits including fruit quality, yield, shelf life, and resistance to biotic and abiotic stress by changing the target genes in specific biochemical and signalling pathways.

Table 1: Genetic improvement of fruit crops using genome editing technologies.

Fruit crops	Gene targeted	Genome editing tools	Function of gene	References
Sweet orange	*CsPDS* gene	CRISPR/Cas 9 sgRNA	Biosynthesis of carotenoid	Jia and Wang (2014)
Apple and Fig	*uidA* gene	ZFNs under heat shock promoter	Reporter gene	Peer *et al*. (2015)
Grape	*MLO-7* in grape	CRISPR/Cas 9	*MLO* – susceptible gene for powdery mildew	Malnoy *et al*. (2016)
Kiwifruit	*AcPDS*	CRISPR/Cas 9	Biosynthesis of carotenoid	Wang *et al*. (2018)
Banana	Five MaGA20ox2	CRISPR/Cas9 each gene's second exon is targeted by two sgRNAs	Biosynthesis of gibberellin	Shao *et al*. (2019)
Walnut	*JrPDS*	CRISPR/Cas9 *JrPDS*is targeted by five sgRNAs	Biosynthesis of carotenoid	Walawage *et al*. (2019)
Pomegranate	*PgUGT84A23* and *PgUGT84A24*	CRISPR/Cas9/two sgRNAs	UDP-dependent glycosyl transferase enzyme biosynthesis	Chang *et al*. (2019)
Grape	*VvPDS*	CRISPR/Cas9 Four sgRNAs with different GC content	Biosynthesis of carotenoid	Ren *et al*. (2016)

Resistance to biotic and abiotic stresses. The main cause for reduced yield and quality of fruits and nuts are pest and disease incidence. In order to start a series of signal transduction and defense pathways involving numerous genes and their byproducts, plant defense recognizes pathogen compounds. On the other side, pathogens attempt to obstruct the pathways leading to the defense response. Citrus canker (*Xanthomonas axonopodis*) susceptibility in Duncan grapefruit is controlled by *CsLOB1*, which has been discovered. Citrus canker resistance levels varied along the lines when CRISPR/Cas 9 was used to specifically mutate *CsLOB1* in Duncan grapefruit (Jia *et al*., 2017). Later, the homozygous Duncan grapefruit plant showed resistance to citrus canker disease after editing of the *CsLOB1* promoter (Peng *et al*., 2017). Similarly, many plant species are known to be susceptible to diseases caused by powdery mildew by *MILDEW – RESISTANCE LOCUS* (MLO) family genes (Kusch *et al*., 2017; Yu *et al*., 2019). Grapevine *VvMLO3* and *VvMLO4* mutations using CRISPR/Cas 9 technology showed that *VvMLO3* allele mutations boosted resistance to powdery mildew in sensitive cultivars (Wan *et al*., 2020). *Erwinia amylovora* interacts with the DspA/E effector and the susceptibility gene *MdDIPM4* to generate fire blight disease in apples. CRISPR/Cas 9-mediated knockout of the susceptibility gene *MdDIPM4* in susceptible cultivars resulted in decreased susceptibility to the disease (Pompili *et al*., 2020). Efforts have been made to increase the grapevine's ability to withstand water stress by using CRISPR/Cas 9 to mutate *VvMYB60*, an ortholog of *AtMYB60* (Dalla *et al*., 2019). It has been demonstrated that *AtMYB60* controls stomatal activity in Arabidopsis in response to ABA and improved ability to withstand drought (Cominelli *et al*., 2005).

Reducing the juvenile period. Due to the long juvenile periods, domestication and breeding of tree species lag behind that of annual and biannual crops.The transition from the vegetative to reproductive phase occurs when the floral integrator genes *FLOWERING LOCUS T (FT), SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1),* and *TERMINAL FLOWER1 (TFL1)* receive signals from internal (phytohormones) and environmental (photoperiod, temperature) variables. The *LEAFY (LFY)* and *APETALA1 (AP1)* floral meristem identity genes are controlled by the floral integrator genes, which result in the floral transition (Liljegren *et al*., 1999; Litt *et al*., 2003). In plants, especially trees, it has been demonstrated that changed expression of some of the important genes controlling flower initiation speeds up the transition to the reproductive phase (Flachowsky *et al*., 2011; Freiman *et al*., 2012). Overexpressing Arabidopsis *LEAFY (AtLFY)* or *APETALA1 (AtAP1)* led to early blooming and fruit development in a citrus interspecific hybrid, which occurred 12 to 20 months after the transformants were transferred to the greenhouse. When the Trifoliate orange (*Poncirus trifoliata*) has the *Citrus unshiu* FLOWERING LOCUS T (*CiFT*) over expressed, it causes early flowering in about 12 weeks after being moved to a greenhouse. (Endo *et al*., 2005). *CENTRORADIALIS (CEN)*, a floral repressor gene, was altered using CRISPR-Cas9 in kiwifruit, resulting in extremely early and continually flowering line (Varkonyi-Gasic *et al*., 2019). Making a perennial plant like the kiwifruit flower all year long has the advantage of allowing for quick breeding cycles and year-round fruit production as opposed to seasonal harvest (Eshed *et al*., 2019). Therefore, reducing juvenility and accelerating the genetic improvement process can be accomplished by employing genome editing to change the expression of the crucial genes governing flower initiation in fruit and nut crops (Callahan *et al*., 2016).

Fruit quality and shelf life. Significant amounts of secondary metabolites, which have both aesthetic and useful properties, are present in fruits and nuts. For instance, the fruit pigments anthocyanin and lycopene have a number of functions, including being antioxidants, anti-inflammatory, and anti-cancer (Khoo *et al*., 2017). These pigments, in addition to carotenoids and chlorophylls, serve as indications for fruit quality and maturity by giving colour to fruits. Genome editing was used to increase the amount of lycopene in tomatoes by encouraging lycopene synthesis while preventing its conversion to α- and β-carotene. Five genes were simultaneously knocked down using CRISPR/Cas 9 namely stay-green 1 (*SGR 1*), lycopeneε-cyclase (*LCY-E*), beta-lycopene cyclase (*Blc*), lycopene β-cyclase 1(*LCY-B1*) and *LCY-B2*and this led to increased lycopene content by 5-folds. By using CRISPR-Cas9 to delete the gene in suspension cells, it has been demonstrated that the L-idonate dehydrogenase (*IdnDH*) gene regulates tartaric acid (TA) biosynthesis in grapevine (Wang *et al*., 2019). Therefore, fruit crops with high quantities of useful pigments and metabolites could be created by genome editing some of the important genes.

Shelf life is a crucial aspect of fruit quality after the harvest of ripe fruits. Ethylene is essential for the ripening and softening of fruit, according to studies on the shelf-life of fleshy fruits, like tomatoes (Wang *et al*., 2019). The shelf life of fruits can be enhanced by inhibiting the biosynthesis of ethylene and signal transduction. Gene expression can be suppressed by either removing the gene or altering the methylation status of the DNA. By employing CRISPR-Cas9 gene editing to eliminate the Banana fruits' shelf life was increased by 40 days compared to the wild type when the *MaACO1* (aminocyclopropane-1-carboxylate oxidase 1) gene, which codes for the enzyme that converts ACC to ethylene, was expressed (Hu *et al*., 2021). Therefore, fruit tree crops can yield fruits with longer shelf life and consequently lower post-harvest losses by inhibiting or altering the methylation state of the main genes involved in the ethylene generation or ripening process or their signalling pathways.

CONCLUSION AND FUTURE SCOPE

Genome editing provides a wide range of potential for crop improvement, particularly fruit and nut trees, that are challenging to improve using traditional breeding techniques because they provide accurate, effective and more rapid genetic changes. Genome editing offers to hasten the breeding of fruit and nut crops, which is particularly necessary to fulfill the rising global demand

under changing climate with less growth resources. CRISPR/Cas system have now been utilized mostly for gene knockdown experiments in fruit and nut crops. CRISPR/Cas 9 has the capacity to make specific changes to genes of interest.

Genome editing will eventually be expanded to target a wide range of genes in order to produce fruit and nut crops with improved production and quality. Additionally, genome editing permits the direct incorporation of introducing new or enhanced traits into popular cultivars that are lacking in one or more, without altering their other characteristics. Crop varieties' wild ancestors have advantageous traits such the capacity to endure biotic and abiotic stressors, improvement in fruit quality, etc. Wild species are thus possible sources for genome editing. The lines produced by genome editing methods can be used directly as a new variety in industrial production or as pre-breeding stock in breeding programmes. Thus, with the development of genome editing, it is now possible to develop superior fruit and nut crops more quickly and with lower danger of off-target impacts.

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Conflict of Interest. None.

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